UNIVERSITY OF GONDAR

COLLEGE OF NATURAL AND COMPUTATIONAL SCIENCES

DEPARTMENT OF BIOTECHNOLOGY

Association of Human Leukocyte Antigen (HLA*) B-27* Polymorphism with Risk of Diabetes Type one among Patients Visiting Gondar University Teaching Hospital

A Thesis Submitted to the Department of Biotechnology for the Partial fulfillment of M.Sc. Degree in Biotechnology

By Nigatu Mengistu

Advisor: NegaBerhane (PhD)

May, 2017

Gondar, Ethiopia.

APPROVAL SHEET

As a thesis advisor, I hereby certify that I have read and evaluated this thesis prepared under my guidance, by Nigatu Mengistu entitled as

Association of human leukocyte antigen (HLA*) B-27* polymorphism with risk of diabetes type one among patients visiting Gondar university teaching hospital

I recommended the paper to be submitted as fulfilling the requirement for the degree of Master of Science in Biotechnology

Dr. NegaBerhane _____ _____

Advisor Signature Date

As members of the board of examiners for the MSc thesis open defense examination, we certify that we have read and evaluated the thesis prepared by Nigatu Mengistu and examined the candidate. We commended the thesis to be accepted as fulfillment for the requirements of the degree of Master of Science in Biotechnology (medical biotechnology).

_____ _____ _____

Chair Person Signature Date

_____ _____ _____

Internal Examiner Signature Date

_____ _____ _____

External Examiner Signature Date

DECLARATION

I, the undersigned, declare that this thesis is my original work. It has never been submitted in any institution and that all the sources of materials used for thesis have been dully acknowledged.

Student Nigatu Mengistu Signature _____ Date_____.

ACKNOWLEDGMENTS

Right from the outset, I would like to thank the supreme Almighty God for provide me the patience and endurance to complete the study. Next I would like to express my deepest heartfelt thanks to my advisor, NegaBerhane. (PhD, Associate Professor), He supported economically to providing expensive. reagents and for his unreserved guidance, supervision, valuable suggestion and intensive help, for the freedom discussion and generous personal encouragement starting from the initial stage to thesis proposal to the final write up.

My special and grateful thanks go to University of Gondar, Department of Biotechnology, for providing me all the resources and facilities used in the study.I wish to express my special thanks to Mr. GashawEbabu for his great help during collecting of samples and work of this study.

Also, I am very thankful to Mr. TekebaSisay, Mr. Wagaw Sendeku, who supported me in the optimization of PCR protocol, arranged primers with suitable proceeded and for they valuable comments during the write up process of Mr. MolalignMedifu for their professional guidance. I gratefully acknowledge TadeleTamru, MeqaninntDagnew, for their help during the laboratory work of this study. ‘

Finally, I am grateful to my sisters and brother for their endless support and care during this thesis as in all stages of my life.

TABLE OF CONTENTES

LIST OF TABLES

List of figure

LIST OF ABBREVIATION

A Adenine

AS Ankylosing spondylitis

ASA Spondyloarthritis.

B Beta value

CI. Confidence interval

DF Degree of freedom

DM Diabetes Miletus.

DNA Deoxyribo Nucleic Acid

DNK Diabetic ketoacidosis

DNTP Deoxyribo Nucleotide Tri Phosphate

EDTA Ethailendiamentertaasetic acid

G Guanine

HIV Human Immune Decency Vires

HLA Human leukocyte anti gene

HLA B Human leukocyte anti gene B

HLA B-27 Human leukocyte anti gene B-27.

IDF International Diabetes Federation

LAD Latent autoimmune diabetes in the adult

MHC Major Histocompatibility complex

NCD Non communicable diseases.

NIDDK National Institute of Diabetes and Digestive and Kidney Diseases ..

OR Odds Ratio

P probability

PCR Polymerase chain reaction

SPSS Statistical package for social science

SSPsingle specific primer polymerase

SNP Single Nucleotide polymorphism

T1D Type one diabetes.

WHOWorld Health Organization

ABSTRACT

Diabetes mellitus is a complex multifactorial and heterogeneous syndrome characterized by hyperglycemia resulting from inadequate insulin secretion or insulin action. Type one diabetes is a chronic inflammatory disease caused by a selective destruction of the insulin-producing β-cells in the islets of Langerhans. It is an important public health problem. It accounts for between 5% and 10% of all diabetes cases. The main genomic region controlling the predisposition to type one diabetes is the Human Leukocyte Antigens (HLA) class I of the major histocompatibility complex. The aim of the present study was to evaluate HLA B-27 polymorphism and risk factor for type one diabetic among patients visiting University of Gondar Teaching Hospital Gondar, North West Ethiopia. Two hundred blood samples were collected from September, 2015 to July,2016 from clinically confirmed type one diabetes mellitus patients (n= 100) and from type one diabetes mellitussero-negative individual (n= 100) that live in the study area by studying of case control method. HLA B-27 polymorphism was detected using PCR-SSP (single specific primer polymerasereaction) techniques. Statistical analyses of valuable data were performed by SPSS (version 20) package. Forty eight percent of study participant's type one diabetes were with in the age range of less than forty years old and 67% were male. Age (P=0.000), study participants were living rural area (p=0.001) were associated risk factor for type one diabetes mellitus disease at 95% CI. The allele frequency in type one diabetes patients study subject (n=100)was 0.67 and the remaining 33.the distribution of HLA-B-27 genotype in cases (p=0.000 and control (p= 0.001) were consistence with the Hardy winberg equation. In type one diabetes mellitus patients study subject.67of the study participantswerehomogenous for HLA B27.In control groups the frequency of *HLA-B27* was 0.25 and the remaining 75 study subjects were negative for the mentioned allele. There was statistically significance association between *HLA-B27genotype and* type one diabetes. (p=0.001, OR=1.370).

Further studies with large numbers of sample size within the same and different population are reasonable in order to consider this polymorphism as a marker for type one diabetes mellitus

.Key Words: β-cells, human leukocyte antigen*B-27*, gene polymorphism, type one diabetes mellitus

1.INTRODUCTION

1.1 Background

Diabetes mellitus is a complex multifactorial and heterogeneous syndrome characterized by hyper glycaemia resulting from inadequate insulin secretion or insulin action (Njolstad*et al.*, 2003). It is one of leading causes of morbidity and mortality in the world, the prevalence of diabetes around the world is increasing rapidly (WHO,2013). Estimated that diabetes resulted in 1.5 million deaths in 2012 as of 2013, 382 million people have diabetes worldwide (Shi *et al.*, 2014).In 2014 and 2015 the International DiabetesFederation (IDF) estimated that diabetes resulted in 4.9 million deaths, (IDF, 2016).

The number of people with diabetes is expected to rise to 592 million by 2035. (IDF, 2014).The highest prevalence rates are found in the North American region (9.2%), followed by the European region (8.4%).More than 80% of diabetic deaths occur in low and middle-income countries In Africa, the prevalence of Diabetes mellitus is increasing and the magnitude of the disease is progressing (Gill *et al.*,2009). In sub-Saharan Africa, over 12million people are expected to have Diabetes mellitus, and 330,000 of these people will die from this disease related complications (Motala *et al.*, 2010).

In Ethiopia, according to World Health Organization estimation, the number of diabetics cases in the year 2000 was 800,000 and this number is expected to increase to 1.8 million by the year2030 (Motala *et al.*,2010). There are four different types of diabetes found throughout the world type 1, type 2, gestational diabetes, and other specific types (Shoback*et al.*,2011).pose the great public health challenge.type1 diabetes mellitus is characterized by loss of the insulin producing beta cells of the islets of Langerhans in the pancreas leading to insulin deficiency (Rother*et al.*,,2007).It accounts for between 5% and 10% of all diabetes cases (Daneman,*et al.*,2006).

Globally, the number of people with Diabetes mellitustype 1 is unknown although, it is estimated that about 80,000 children develop the disease each year (*Chiang, etal.2014*).

The subsequent lack of insulin leads to increased blood and urine glucose. The classical symptoms are polyuria, polydipsia, and polyphagia and weight loss. If left untreated, diabetes can cause many complications (WHO. 2013).Acute complications include diabetic ketoacidosis and non ketotic hyperosmolar coma.(Kitabchi, *et al*., 2009)

Serious long-term complication includes cardiovascular disease, stroke, chronic kidney failure, foot ulcers, and eyes. Several pathogenic processes ranging from autoimmune destruction of the β-cells of pancreas to abnormalities that result in resistance to insulin are involved in the development of diabetes. Furthermore, complications may arise from low blood sugar caused by excessive insulin treatment complications from poorly managed type 1 diabetes mellitus may include cardiovascular disease, diabetic neuropathy, and diabetic retinopathy, among others. However, cardiovascular disease (Devaraj, 2006) as well as neuropathy may have an autoimmune basis, as well. Women with type 1 diabetes mellitus have a 40% higher risk of death as compared to men with type 1 diabetes mellitus (Huxley, 2015).

Moreover, the difference between the Epidemiologic patterns of type one diabetes mellitus by demographic, geographic, biologic, cultural and other factors in populations are presented to gain insight about the etiology, natural history, risks and complications of type one diabetes mellitusdata on to one region to another could largely explain the release of the autoimmunity is stimulated under the influence of one or more environmental factor (Kida, 1999).There is a link between cognitive deficit and diabetes.

Similar to other autoimmune diseases, the etiology of type one diabetes millets remains obscure but develops on a genetically susceptible background and also involves a variety of factors, ranging from immune deregulation to environmental triggers. In addition, Genetic variants (alleles) in the highly polymorphic human leukocyte antigen (HLA) on chromosome 6p21.3 can lead to functional Differences in how fragments of protein are presented to the immune system in the early 1970s,several group investigated(Singal,1973) and found associations with type one diabetes millets.

HLA class I Human leukocyte antigen-B27 is a major his to compatibility complex class I molecules that is strongly associated with ankylosing spondylitis and related sero negative spondyloarthritis. Ankylosing spondylitis is associated with B-27 with a relative risk of 95 which is the highest among all HLA disease associations. The association with HLA-B27 with AS was first reported in 1973 (Brewerton, 1973) and confirmed with related SpA later by many other investigators.

It is currently obvious that the strongest genetic susceptibility to a predisposition is allotted to the insulin dependent diabetes mellitus alleles located in the HLA locus of the chromosome *6p21* (Akerblom, *et al.*,1997). It is mainly HLA alleles that present a high risk of contracting the disease compared to non-HLA alleles (Dorman, 1997).

1.2. STATEMENT OF THE PROBLEM

In Ethiopia, Diabetes mellitus is one of the major health problems resulted in many disability and death. This has several consequences for the country's economy which has impact on achievements of sustainable growth and developments based on life cost.(WHO,2013).

The risks progression of type one diabetes has been thought to be partly accounted by multiple genes including certain HLA genotypes and environmental factors. The genetic factors are likely to involved several genetic alterations in immune mediated as well as molecules involved in mechanisms of cytoadherence.

Understanding the key genetic determinants responsible for type one diabetes susceptibility is critical to prevent frequent disease severity and for developing better therapies vaccine strategy. For this purpose a case control molecular based study is important. As far as our knowledge goes such types of molecular study has not been done before in the study area. So this study was organized to fill this research gaps and to collect molecular based information.

1.3. Significance of the study

The knowledge gained since 1973, using molecular genetics approaches has produced undisputed evidence about polymorphism associated with out immune disease like type one diabetes disease and their complex interactions. Through human gene study different genetic factors were found that contribute to the variability of diabetes.

Molecular analysis of candidate gene will help to determine some of the mechanisms involved in susceptibility to type one diabetes. To understand how to combine type one diabetes efficiently, it is necessary to know the relative importance of factor that contribute to the striking variation among human in their experience of diabetes mellitus.

It is important that case control association studies for type one diabetes be carried out on carefully defined sets of patients and controls to obtain scientific based information about the factors that are related to type one diabetes pathogenesis. In type one diabetes affected areas, putting genetic and environmental factor in to perspective will inform the design and interpretation of intervention studies aimed at reducing the burden of type one diabetes disease and will help rationalize research priorities. Obtaining essential data regarding the frequency of HLA B-27 gene polymorphism and associated risk factors will help to improve the effort being done to reduce and prevent diabetic one in the region specifically and at national level at large. Moreover, such type of molecular analysis studies will aid in screening high risk diabetic one patients early before the onset of the disease so as to have better treatment out comes.

.2. OBJECTIVE

2.1. GENERAL OBJECTIV

The general objective of this study was to assess the association of HLA *B-27* polymorphism and risks of type-one diabetes milieus

2. 2.SPECIFIC OBJECTIVES

The specific objectives of this study were to:-

- Determine the frequency of HLA B-27 allele amongtype one diabetes mellitus patients in the study area.
- Determine the proportion of HLA B27 gene polymorphism with in the study population.
- Assess the effects of HLA B27 gene polymorphism and different demographic factors on the risk of type one diabetes mellitus

3. LITERATURE REVIEW

3.1 DIABETESMELLITUS.

Diabetes is a serious, chronic disease that occurs either when the pancreas does not produce enough insulin, or when the body cannot effectively use the insulin it produces. Insulin, is a hormone produced by the pancreas, controls the blood glucose level by regulating the production and storage of glucose. In diabetes there may be a decrease in the body's ability to respond to insulin or a decrease in the insulin produced by the pancreas which leads to abnormalities in the metabolism of carbohydrates, proteins and fats, resulting hyperglycemia. (Seltzer *et al*., 1992) The prevalence of diabetes is higher in minority groups and among those who are socio-economically disadvantaged, but the reason for that has not been given (Chang, *et al.,* 2010).There are four major classifications of diabetes mellitus, namely:-type one diabetes, (T1D),type two diabetes,(T2D), gestational diabetes (GD),other type (type IV)

3.1.1. Type I

Type 1 diabetes mellitus (T1D) is one of the most common autoimmune diseases with several million people already affected around the globe. It can occur at any age, but is most commonly diagnosed from infancy to the late forties (Daneman, 2006).

It is characterized by an absolute loss of insulin secretion, and results from an autoimmune process that destroys insulin producing ß cells within the pancreatic islet. Similar to other autoimmune diseases, the etiology of Type one diabetes remains obscure but develops on a genetically susceptible background and also involves a variety of factors, ranging from immune deregulation to environmental triggers. Genetic predisposition coupled with viral agents and possibly chemical agents. (Chiang *et al*., 2014)

Type one diabetic is subdivided to three categories from the etiological point of view such as Autoimmune, Idiopathic and Double. The autoimmune group is represented by type 1A, which is polygenic and it is the most frequent type of this disease accounting 80-90% of all type one diabetes millets cases(Chiang *et al.,* 2014)

Idiopathic also called Type 1B, it has all the clinical features of type 1A, but the autoimmune component is not detected. Double (type 1 plus type 2) diabetes has been proposed when we have the type 1A (autoimmunity) plus type 2 (obesity, insulin resistance, dyslipidemia) diabetes characteristics in the same individual. There has been reports in the literature that HLA (Human Leucocytes Antigen) DR3 and DR4 loci (DR3 and DR4) are associated with high risk of T1DM (*Bluestone et al.,*2010)

However, as far as our knowledge goes, there has not been any report in the literature which describes the association of *HLA-B27* polymorphism and risk of T1D in the world. Therefore, this is the first study that attempts to see whether there will be association of *HLA-B27* gene polymorphism and risk of T1D.

3.1.2. Type II

Type two diabetes mellitus characterized by insulin resistance, which may be combined with relatively reduced insulin secretion (Shoback*et al.,* 2011) .The defective responsiveness of body tissues to insulin, is believed to involve the insulin receptor. This type is the most common type of diabetes mellitus. It is predominant forms of diabetes and accounts for at least 90% of all causes of diabetes mellitus (Gonzalez *et al.,* 2009).

Most patients with this form of diabetes are obese, and obesity itself causes some degree of insulin resistance. Patients who are not obese by traditional weight criteria may have an increased percentage of body fat distributed predominantly in the abdominal region. Ketoacidosis seldom occurs spontaneously in this type of diabetes; when seen, it usually association with the stress of another illness such as infection. This form of diabetes frequently goes undiagnosed for many years because the hyperglycemia develops gradually and at earlier stages is often not severe enough for the patient to notice any of the classic symptoms of diabetes. Nevertheless, such patients are at increased risk of developing macro vascular and micro vascular complications (Buehler *et al.,* 2013).

Whereas, patients with this form of diabetes may have insulin levels that appear normal or elevated, the higher blood glucose levels in these diabetic patients would be expected to result in even higher insulin values had their β-cell function been normal. It also life style factors and genetics (Risérus*et al.,* 2009). A number of lifestyle factors are known to be important to the development of this disease, including obesity (body mass index of greater than 30), due to lacks of physical activity, a lack of exercise is believed to cause 7% of cases (Lee, 2012).poor diet, stress, and urbanization.

Dietary factors influence the risk of developing type two diabetes mellitus. Consumption of sugar-sweetened drinks in excess is associated with an increased risk. (Malik, 2010) .The type of fats diet is also important, with saturated fats and trans fatty acids increasing the risk and polyunsaturated and monounsaturated fat decreasing the risk eating lots of white rice also may increase the risk of diabetes (Hu EA, 2012).

During periods of illness or surgery, individuals who usually control their type II diabetes with diet, exercise and oral agents, may require insulin injections. In some individuals oral agents fail to control hyperglycemia and insulin injection is required .The complications of type II result in disruption of lifestyle, psychosocial adjustment and health care expenses. It is frequently treated with, self-monitoring of blood glucose and hypoglycemic agents/insulin. (Lewis *et al.,* 1996)

3.1.3. Gestational diabetes mellitus

Gestational diabetes mellitus is commonly occurs during pregnancy, usually in the second or third trimester, as a result of hormones secretion by the placenta, which prevents the action of insulin. This is also resembles type II diabetes in several respects, involving a combination of relatively inadequate insulin secretion and responsiveness. It happens about 2–10% of all pregnancies and may improve or disappear after delivery (U.S. Department of Health and Human Services (2014).But, after pregnancies approximately 5–10% of women with gestational diabetes are found to have diabetes mellitus, most commonly type 2.

Gestational diabetes is fully treatable, but requires careful medical supervision throughout the pregnancy. Management may include dietary changes, blood glucose monitoring, and in some cases insulin may be required (National Diabetes Statistics, 2011).However, untreated gestational diabetes can damage the health of the fetus or mother risks to the baby include macrodome (high birth weight), congenital cardiac and central nervous system anomalies, and skeletal muscle malformations. Increased fetal insulin may inhibit fetal surfactant production and cause respiratory distress syndrome. Hyperbilirubinemia may result from red blood cell destruction. In severe cases, prenatal death may occur; most commonly as a result of poor placental perfusion due to vascular impairment the risk factors include immediate family members with the disease and presence of islet cell antibodies (Royle*et al.,* 1992).

3.1.4.Other types (type IV)

This type of diabetics occurs where the disease is associated with other conditions like pancreatic disease, hormonal disorders, and drugs such as glucocorticoids and estrogen-containing preparations. These drugs may not, by themselves, cause diabetes but they may precipitate diabetes in persons with insulin resistance (Pandit*et al.,* 1993).

In such cases, the classification is ambiguous, as the primacy of beta cell dysfunction or insulin resistance is unknown. Certain toxins such as Vacor (a rat poison) and pentamidine can permanently destroy pancreatic beta cells (Espost*iet al.,* 1996).

Depending on the ability of the pancreas to produce insulin, the patient may require by oral agents or insulin. Diseases associated with excessive secretion of antagonistic hormones can cause diabetes. There are many drugs and hormones which can impair insulin action. Examples include nicotinic acid and glucocorticoids (yajnik*et al.,* 1992).

3.2. Causes

The cause of type 1 diabetes is unknown (WHO. 2016) a number of explanatory theories have been put forward, and the cause may be one or more of the following: include genetic factors, immunologic factors, environmental factors and infectious agents. (Knip*et al.*,2005).

3.2.1. Type I

3.2.1.1 Genetic factors

People do not inherit type I diabetes mellitus itself, but they inherit a genetic predisposition towards developing type I diabetes mellitus. This genetic tendency has been found in people with certain HLA. HLA is a cluster of genes that is located in the MHC Class I region on chromosome 6, at staining region *6p21*. Certain variants of this gene increase the risk for decreased histocompatibility characteristic of type 1.responsible for transplantation of antigens and other immune processes. The risk of developing type I diabetes is increased three to five times in people who have one of these two HLA types (Bluestone, 2010).

3.2.1.2. Immunologic factors

People with type I diabetes have an auto-immune response, and this auto-immune response is an abnormal response in which antibodies are directed against normal tissues of the body responding these tissues as if they are foreign. Auto-antibodies against islet cells and against endogenous (internal) insulin have been detected in people at the time of diagnosis and even several years prior to the development of clinical signs of type I diabetes (Smeltzer*et al.*,1992).

3.2.1.3. Environmental factors

Environmental factors can influence expression of type one .It has been proposed that certain viruses or toxins may precipitate the auto-immune process that leads to beta cell destruction, and the events that lead to beta cell destruction are not fully understood (Knip*et al.*,2005).

3.2.1.4. Infectious agents

Viral infections may cause type one diabetes mellitus.Itis a virus-triggered autoimmune response in which the immune system attacks virus-infected cells along with the beta cells in the

pancreas.(Fairweather*et al.*, 2002) Several viruses have been implicated, including enter viruses (especially coxsackievirus B), cytomegalovirus, Epstein–Barr virus, mumps virus, rubella virus and rotavirus(Petzold*et al.*, 2015).

3.2.1.5. Chemicals and drugs.

Some chemicals and drugs selectively destroy pancreatic cells. Pyrinuron (Vacor), a rodenticide introduced in the United States in 1976, selectively destroys pancreatic beta cells, resulting in type 1 diabetes after accidental poisoning (Thayer *et al.,* 2012).Streptozotocin (Zanosar), an antineoplastic agent, is selectively toxic to the beta cells of the pancreatic islets. It is used in research for inducing type 1 diabetes on rodents (Wu *et al.,* 2015).and for treating metastatic cancer of the pancreatic islet cells in patients whose cancer cannot be removed by surgery (Brentjens*et al.,* 2001) Other pancreatic problems, including trauma, pancreatitis, or tumors (either malignant or benign) can also lead to loss of insulin production.

3.2.2. Type II

The exact mechanisms that lead to insulin resistance and impaired insulin secretion in type II diabetes are unknown. Genetic factors are said to play a role in the development of insulin resistance. In addition, the following risk factors are associated with the development of type II diabetes: age, obesity, stress, depression, family history and ethnic group (Bain *et al.*,2001). Signs and symptoms present as a result of hyperglycemia (excessive sugar in the blood)there is an increase in urine output (polyuria) which results from the glycosuria (glucose in urine) secondary to hyperglycemia. Patients experience increased thirst (polydipsia) which is secondary to osmotic dieresis and hyper osmolality. Increased appetite (polyphagia) results because of cellular starvation, and decreased storage of calories.

Weight loss in the presence of polyphagia is due to the ineffective metabolism of carbohydrate, protein and fat. Weakness and lethargy are experienced as a result of inadequate energy production (Phipps *et al.,* 1987). Fatigue is another symptom of diabetes mellitus. Wounds heal poorly. They take a long time to heal due to poor blood circulation to the lower extremities. Vaginitis may be an early complaint in females. The person may also complain of blurred vision

(Lewis *et al.*, 1996). Dry mucous membranes, dry skin, tachycardia and nausea may also manifest if the patient is unable to take in enough fluids to replace the losses through osmotic dieresis.

The signs of hypoglycemia (a deficiency of sugar in the blood) are most commonly seen as side effects of insulin therapy or with the use of oral hypoglycemic agents in patients with diabetes mellitus. Signs such as sweating, trembling and shakiness, which result from increased sympathetic stimulation, cause considerable discomfort. Patients can also experience palpitations, nervousness, hunger, faintness, weakness, irritability, headaches, visual disturbance, marked personality changes and confusion. A coma and convulsions can follow (Phipps *et al* 1987).

3.3. Normal physiology

Insulin is secreted by beta cells in the islets of Langerhans in the pancreas. When a meal is eaten, insulin secretion increases, and moves glucose from circulation into muscle, liver and fat cells. Insulin stimulates storage of glucose in the liver and muscle; it also enhances storage of dietary fat in adipose tissue and accelerates the transportation of amino acids derived from dietary protein into cells. Insulin further inhibits the breakdown of stored glucose, protein and fat. In normal conditions insulin is released continuously into the blood stream. The activity of released insulin lowers blood glucose and facilitates a stable, normal glucose range of approximately 3.9 to 6.7 mmol/l. (McCance, *et al* 1997).

During fasting periods (between meals and overnight) there is a decreased release of insulin and increased release of glucagon. Glucagon counters the effects of insulin because it stimulates the release and breakdown of glycogen from the liver and thereby increases blood glucose levels. The net effect of the balance between insulin and glucagon levels is to maintain a constant level of glucose in the blood (Smeltzerandbare ,1992).

3.4. Management and control of diabetes mellitus

The focus of the management of diabetes is maintaining a healthy lifestyle by following the correct diet, exercising, and taking medication as prescribed.(Elliott *et al.,* 1996).

3.4.1. Diet

Diet constitutes the foundation of diabetes management. The nutritional management of the patient with diabetes is geared towards the following: provision of all the essential food constituents meeting energy needs maintenance of an ideal weight decrease of elevated blood lipid levels achievement of blood glucose levels close to normal The diet that is prescribed for diabetic patients as well as the one that is restricted for them will be discussed. Type II diabetes is treated by diet and exercise, and only when elevated glucose levels persist are supplements of oral agents as well as insulin injections given (Chang, *et al.*, 2002).

3.4. 2.1. Dietary restrictions

People with diabetes found the most difficult component of their self-care regimen to be adhering to a healthy diet. People with diabetes were reported to be more resistant to dietary change when compared to people with other chronic diseases *(*Bare *et al.*, 1992).reported in their research study that diabetics seem to fail in keeping to an appropriate diet for various reasons.

The patient may overeat to cope with stress and negative feelings. When the patient eats away from home, it makes it hard for him/her to control what and how much is eaten. The patient may not resist temptation and intense cravings. Deprived feelings, social events (Bare, 1992) also indicate that social support is important for patients with chronic diseases as it promotes adherence to self-care, thereby achieving better metabolic control. They further indicate that emotional support might be a motivating factor in improving adherence to health recommendations. (BorcJohnsen, *1984).*

Diabetic patients indicated that food restriction is the main reason for failure to adhere to their diet. The reason given is sound because people say the food is nice or well-cooked if it is salty, fried or sweetened, but if the food is boiled or unsalted, people often say that the food is not

palatable because they need fried, salted food, cakes and sweet drinks. Some patients have a tendency of following a high sodium diet whereas they have been advised to follow a low sodium diet, and others drink tea with sugar and not sweeteners. (Shehadeh,*2001).*

They are doing this when they think that they are not seen by health care providers, forgetting what the end-result will be. Dietary restrictions reduce the social activities of some of the patients, as they no longer attend parties or women's clubs, to mention but a few, for fear of temptation. At social gatherings they eat whatever is available and in the end their blood glucose level is not controlled and they may end up with hyperglycemia. In the case of hypertensive patients, their blood pressure remains elevated (Bare *et al.*, 1992).

The diet that is forbidden to diabetic patients is the one that is eaten at parties, for example, cakes, cold drinks, sweets, fatty foods in the form of meat as well as alcoholic beverages. (Bare, 1992) indicate that alcohol ingestion needs to be completely restricted by diabetic patients because of the danger of hypoglycemia when alcohol is taken on an empty stomach. Alcohol consumption may lead to excessive weight gain due to the high calorie content of alcohol. The patient on diabetes may experience headaches, warmth, nausea, vomiting, sweating and thirst following alcohol consumption.

Excessive alcohol intake may impair a person’s ability to recognize hypoglycemia and to follow a prescribed meal plan in order to prevent hypoglycemia. Reduction of fat intake is stressed in order to reduce risk factors such as elevated serum cholesterol levels which are associated with the development of coronary heart disease.

3.4. 2.2. Prescribed diet

The diet that should be followed by diabetic patients is discussed below, and reference is made to the importance of eating three meals per day and having snacks in between meals to prevent hypoglycemia. The diet that is recommended for diabetic patients should comprise of the following:- Carbohydrates like brown bread, maize meal, cereals and potatoes, because they add

bulk to the diet, making it difficult to over-eat on these foods even when eating to satisfy the appetite. Food rich in fiber, e.g. legumes, oats, soya products, vegetables and some fruits - a high fibre diet plays a role in lowering cholesterol in the blood, and improving blood glucose levels, thus decreasing the need for exogenous insulin.

There are two types of fibre, soluble and insoluble. Soluble fibre lowers blood glucose and lipid levels. It forms a gel in the gastro-intestinal tract, which slows the emptying of the stomach. The result is a slower rate of glucose absorption from the food, and has a potentially glucose-lowering effect. Insoluble fibre increases stool bulk and prevents constipation (Bare*et al.*, 1992).

A low-fat diet is recommended to reduce the risk of elevated serum cholesterol levels, and reduce weight. Vegetables can be eaten raw or boiled (without the addition of sugar, salt or butter). Spices, herbs and salt meant for hypertensive patients, that are available at selected supermarkets, should be used instead of ordinary salt. These reduce the accumulation of fluids in the body caused by the intake of ordinary salt. Snacks such as fruits , proteinandvitamin should be part of the diet and should be taken between meals to prevent hypoglycemia and to prevent the patient from eating full meals frequently which can lead to weight gain. A glass of low fat milk or yoghurt should be taken per day. Although they contain simple sugars, their avoidance is inappropriate (Delport*et al.*, 2002).

The use of a moderate amount of table sugar is gaining wider acceptance, provided the patient can maintain adequate blood glucose levels, blood lipid levels and weight control. For some patients, a more liberal use of simple carbohydrates can be a major factor in promoting adherence to a meal plan .Patients should drink pure juice and diet cold drinks. Sweeteners should be used to sweeten drinks such as tea and coffee. There are two types of sweeteners, namely nutritive and nonnutritive sweeteners.

Nonnutritive sweeteners are calorie-free and can be taken in unlimited quantities; whereas nutritive sweeteners should be taken in small quantities as large quantities cause hyperglycemia indicate that diabetic patients should maintain their ideal weight.

It is therefore important that overweight patients adhere to their diet in order to reduce weight. They can adhere to their prescribed diet with the help of adifaxcapsules, which prevent weight gain while the patient is following the normal diet.

This may ensure adherence to the recommended diet as the patient may eat whatever she/he likes, at any time, but still attain the ideal body weight. Another drug said to promote and maintain weight loss is xenical. If taken for two years, it prevents weight gain as indicated by the report. Long-term adherence to the meal plan is one of the most challenging aspects of diabetes management. For those who have lost weight, maintaining the weight loss is often difficult. In order to assist these patients to incorporate new dietary habits into their lifestyles, group support and on-going nutrition counseling is encouraged. The meal plan for all diabetic patients must take into consideration the patient's food likes and dislikes lifestyle, usual eating times, and ethnic and cultural background (Bare *et al.,* 1992).

3.4. 2.3. Exercise

Exercise is considered an essential part of diabetic management. It contributes to the reduction of weight and cholesterol level. Exercise is important because of its effect on lowering blood glucose and reducing cardiovascular risk factors.(Peter *et al.,* 2007) It increases the uptake of glucose by the body muscles and improves insulin utilization. Patients should be encouraged to exercise daily at the same time and for the same amount to maintain constant, normal blood glucose levels. Patients need to consult the health care personnel before resuming with exercise to give them advice on when to exercise and when not. This is important because exercising with elevated blood glucose levels will cause increased secretion of glucagon’s, growth hormone and catecholamine’s. The liver will then release more glucose, resulting in an increase in blood glucose.(Peter *et al.,* 2007).

Exercise should therefore not be performed until blood glucose levels are under 14mmol/L. Patients should monitor blood glucose levels before, during and after exercise to determine the effect exercise has on the blood glucose level at particular times of the day. They should be made

aware of the possibility of exercise-induced hypoglycemia, which may occur after exercise (Smeltzer, 1992).

Patients on insulin should take a fruit or snack before engaging in moderate exercise to prevent hypoglycemia. The fruit or snack taken need to be deducted from the regular meal plan. The patient may eat a snack at the end of a strenuous exercise session, and may reduce the dosage of insulin that is peaking at the time of exercise to avoid post-exercise hypoglycemia. Exercise can be in the form of walking, swimming, aerobics, playing soccer or participating in athletics (Helen *et al.*,2004).Physical activities such as gardening and housework are also regarded as exercise as these also assist in facilitating the effect of insulin and promote a feeling of general well-being which reduces the stress caused by diabetes

3.4. 2.4. Treatment and Prevention of Diabetes:-

At the present time, there is no way to prevent T1D. Lifelong insulin injections are the only available treatment for the disease. Thus, genetics does not currently play a role in the management or prevention of T1D. Although a cure for T1D is currently unavailable, several large multi-national investigations have been designed to evaluate a variety of primary and secondary disease interventions (NIDDK, 2015). Insulin therapyis usually given by injection just under the skin but can also be delivered by an pump. Some researchers believe it might be prevented at thelatent autoimmune stage, before it starts destroying beta cells.(NIDDK. 2016).

3.4. 2.5. Immunosuppressive drugs

Cyclosporine A, an immunosuppressive agent, has apparently halted destruction of beta cells (on the basis of reduced insulin usage), but its kidney toxicity and other side effects make it highly inappropriate for long-term use.Anti-CD3 antibodies, including teplizumab and otelixizumab, had suggested evidence of preserving insulin production (as evidenced by sustained C-peptide production) in newly diagnosed type 1 diabetes patients. A probable mechanism of this effect was believed to be preservation of regulatory T cells that suppress activation of the immune system and thereby maintain immune system homeostasis and tolerance to self-antigens. The duration of the effect is still unknown, however. In 2011, Phase III studies with otelixizumab and

teplizumab both failed to show clinical efficacy, potentially due to an insufficient dosing schedule.(Bio space. Retrieved 29 November, 2011)

An anti-CD20 antibody, rituximab, inhibits B cells and has been shown to provoke C-peptide responses three months after diagnosis of type 1 diabetes, but long-term effects of this have not been reported.

3.5. Human leukocyte antigen production and function

The human leukocyte antigen (HLA) system is a gene complex encoding the major histocompatibility complex (MHC) proteins in humans. These cell-surface proteins are responsible for the regulation of the immune system in humans. The HLA gene complex resides on a 3 Mbp stretch within chromosome 6p21 (Beck *et al.*,1999). HLA genes are highly polymorphic, which means that they have many different alleles, allowing them to fine-tune the adaptive immune system

The HLA system is studied from various viewpoints including, organ transplantation, population genetics, disputed parentage, disease association studies and to answer basic questions of immune biology. Genes in this complex are categorized into three basic groups: class I, class II, and class III. Humans have three main MHC class I genes, known as HLA-A, HLA-B, and HLA-C. The proteins produced from these genes are present on the surface of almost all cells. On the cell surface, these proteins are bound to protein fragments (peptides) that have been exported from within the cell. MHC class I proteins display these peptides to the immune system. If the immune system recognizes the peptides as foreign (such as viral or bacterial peptides), it responds by triggering the infected cell to self-destruct (Gill *et al.*,2009)

There are six main MHC class II genes in humans: HLA-DPA1, HLA-DPB1, HLA-DQA1, HLA-DQB1, HLA-DRA, and HLA-DRB1. MHC class II genes provide instructions for making proteins that are present almost exclusively on the surface of certain immune system cells. Like MHC class I proteins, these proteins display peptides to the immune system.

The proteins produced from MHC class III genes have somewhat different functions; they are involved in inflammation and other immune system activities. The functions of some MHC genes are unknown. HLAs have other roles. They are important in disease defense. They are the major cause of organ transplant rejections. They may protect against or fail to protect (if down-regulated by an infection) against cancers. (Galbraith *et al.*,1991).

Mutations in HLA may be linked to autoimmune disease (examples: type I diabetes.) HLA genes have many possible variations, allowing each person's immune system to react to a wide range of foreign invaders. Some HLA genes have hundreds of identified versions (alleles), each of which is given a particular number (such as HLA-B27). Closely related alleles are categorized together; for example, at least 40 very similar alleles are subtypes of HLA-B27.

These subtypes are designated as HLA-B*2701 to HLA-B*2743.(Khan *et al.,2010).* is a class I surface antigen encoded by the B locus in the major histocompatibility complex (MHC) on chromosome 6 and presents antigenic peptides (derived from self and non-self antigens) to T cells. HLA-B27 is strongly associated with ankylosing spondylitis (AS), and other associated inflammatory diseases referred to as spondyloarthropathies.. Diseases associated with the HLA-B27 subtype can be remembered with the mnemonic PAIR, and include Psoriasis, Ankylosing spondylitis, Inflammatory bowel disease, and Reactive arthritis a small group (<0.5%) of people infected with HIV are able to remain symptom-free for many years without medication. These long-term no progresses appear to be slightly more common among people who are HLA-B27 positive(Blajchman, 1973).

3.6. Disease associations

The relationship between *HLA-B27* and many diseases has not yet been fully elucidated. Though it is associated with a wide range of pathology, particularly seronegativespondyloarthropathy, it does not appear to be the sole mediator in development of disease. For example, while 90% of people with ankylosing spondylitis (AS) are *HLA-B27* positive, only a fraction of people with *HLA-B27* ever develop AS. People who are *HLA-B27* positive are more likely to experience early onset AS than *HLA-B27* negative individuals (Feldtkeller*et al., 2003).*

4. MATERIAL AND METHODS.

4.1. Study area.

Gondar town is found in North West Ethiopia 727Km away from Addis Ababa. The town has 12°C 36' North latitude and 37°C 28'East longitude with an elevation of 2133 meter above sea level. Average maximum and minimum temperature is 29°C (in March and May) and 10°C (in January and December) respectively. The mean relative humidity for an average year is recorded at 55.7% and monthly bases it ranges from 40% In January to 79% in July (central statistics Agency 2011).

4.2. Ethical Consideration

For this study, ethical clearance was obtained from the research and ethical committee of college of natural and computational science, informed consent was taken from all sturdy participants(**Annex1 P-42)**

4.3. Study Design.

The study design was a case control study design

4.4. Sample Collection.

Three milliliter of one hundred blood samples were collected from clinically confirmed T1DM patients who visited Gondar university teaching hospital from September to July, 2016using ethylene diamante tetra acetic acid (EDTA) coated test tubes. All the samples were collected from T1D follow up patients. In the meantime age and sex matched hundred blood samples were collected from type one diabetes sero negative individuals (who had no T1D case or history) who are being confirmed of having no major metabolic diseases.

The samples were transported to laboratory by using ice box and the laboratory work was done at molecular biology laboratory of department of biotechnology from September to July, 2016. Demographic information such as age, sex, educational status, occupation, and residence of the

patients and controls were collected using semi structured questioner and tabulated for associational studies and to determine the risk.

Isolation OF Genomic DNA

Genomic DNA was isolated from EDTA anti coagulated peripheral blood according to standard proteinase K digestion and phenol chloroform extraction method (Roe *et al,* 1995).

Protocols:-

Frozen blood samples was thawed

↓↓

1ml of whole blood was added in 2.5 ml eppendroof. andequal volume of 0.8M 1Xsodium citrate buffer was mixed gentlyand centrifuge 10,0000 RPM for 5 mantis in cooling centrifuge(4°C)

↓↓

1ml of supernatant was discarded.

↓↓

to the eppendroof 1ml of 0.8M 1Xsodium citrate bufferwas added was centrifuge 10,000 RPM for 5 mantis in cooling centrifuge(4°C) for further purification .then

↓↓

375 µl of 0.2M Sodium citrate was added mix gently briefly vortexed .lt was followed by the addition of 250 µl 10 % of sodium dodosilsulpfhat and 5 µl proteinase K (20mg/ml water) then vortexad briefly and incubated for 1 hour for 55 °C

↓↓

0 µl mixtures of phenols(60 µl)/chloroform (57.6 µl)/ isoamyl alcohol (2.4 µl)was added to eppendroof and then vortexed 30 seconds then centrifuge 10,000 RPM for 5 mantis then

↓↓

The upper layer was separately transferred in a new eppindrooftube then add absolute ethanol (00%) incubate over night for deep freeze then centrifuge 5, 0000 RPM for 5 mantis

↓↓

Then discard supernatant and add the pellet dissolved in 100 µl of tries- EDTA..

PREPARATIONS OF AGAROSE GEL

0.5ml of (1x) TEA buffer was taken in the beaker

↓↓

and addAGAROSE mix gently

↓↓

(0.50gm0f agaros was added in 50 ml of 1x TAE and then the solution was boiled (using microwave oven) till all gel particle was dissolved-

↓↓

-the solution was allowed to cool water bath 50°C --60°C

↓↓

Casting of the horizontal agaros gel.

↓↓

The gel was assembled to casting tray and the comb was positioned at its

↓↓

The agaros solution was poured in to the gel tray and kept at room temperature

↓↓

The comb was carefully remove

↓↓

and the gel replaced in electrophoresis chamber

↓↓

The chamber was filled with TAE electrophoresis buffer till it reached 3-5mm the surface of over the surface of the gel.

↓↓

LODING and running

↓↓

DNA (3 µl) in aggaros gel was mixed with bromophnol blue in the ratio of 3:1 and loaded in

the wells of the 0.8% agaros gel.

↓↓

The cathode was connected to the well side of the unit and the anode to the other side

The gel was run 60v till the bromo phenol blue tracing dye migrated near to the other end.

The quality of DNA was observed by staining the gel with ethidium bromide (0.5 µl)and visualized underUvtrancellumitantor (fig.1).

Genomic DNA from cases group 67 male and 33 female individuals also in control groups59 male and 41 female individuals genomic DNA isolated from whole blood by phenol chloroform method (Roe *et al.,* 1995). The quantity and quality of the isolated DNA was analyzed by spectrophotometer and agarose gel electrophoresis respectively before further processing.

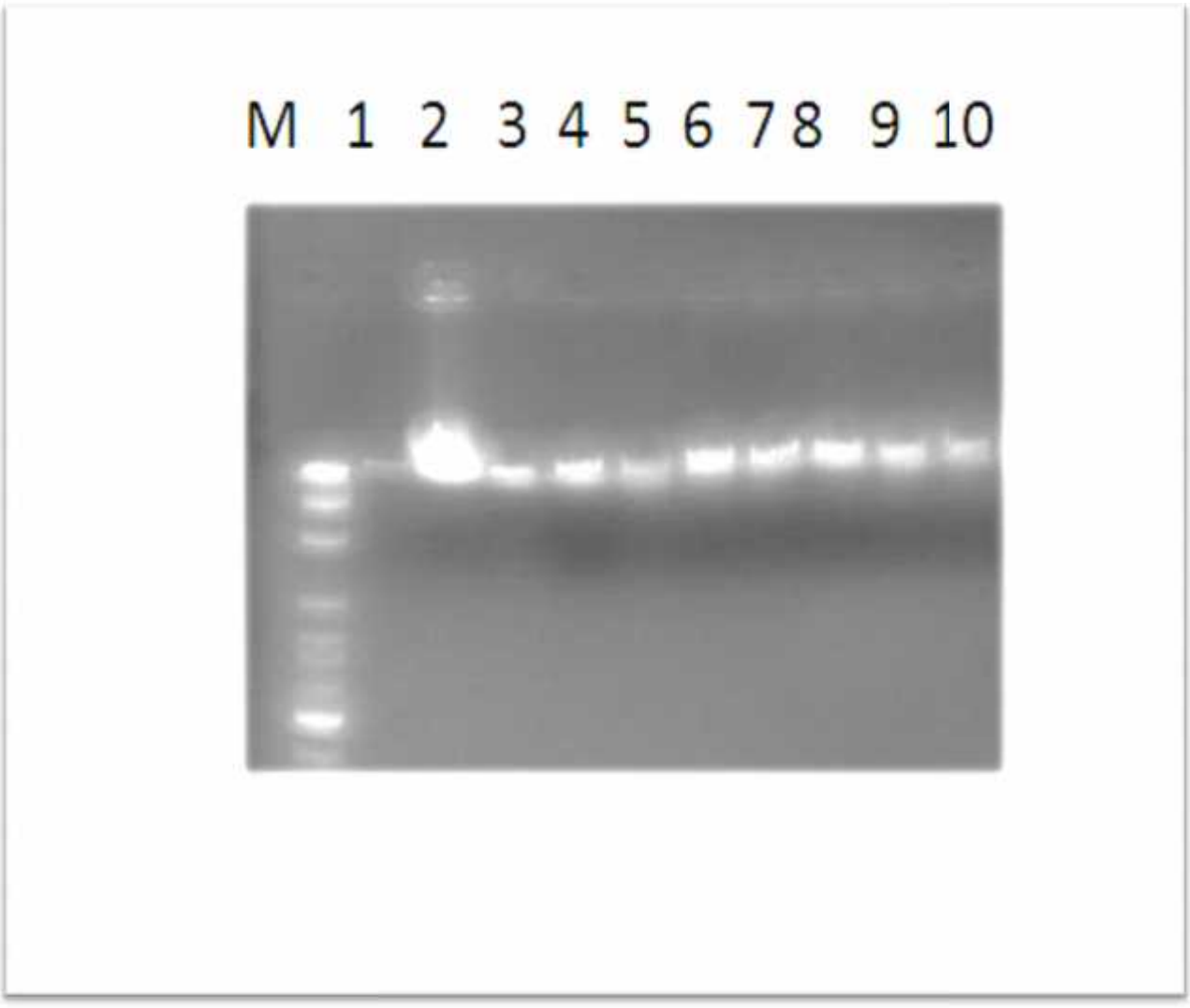

Figure 1.Agaros gel electrophoresis of genomic DNA isolated from whole human blood M: 100bp molecular weight marker.

4.6. Detection of HLA *B-27* antigen polymorphism.

Human leukocyte antigen B -27 gen polymorphism was detected by using PCR-SSP (single specific primer polymerase reaction) techniques. This was done be amplifying a 101-153 base pair (bp) of DNA fragments flanking the polymorphic site using primer pair, HLA-27: forward CGTGGACGACACGCT-3'Reverse-5'CTCGGTCAGTCTGTCTCGGGCCTT3',ControlprimersequencesHGH-Forward-5'-TGCCTTCCCAACCATTCCCTTACCATT-3'HGH Reverse 5'TCTGTTGTGTTTC-3.' (Sylvain, 2004) amplify the target 439 HGHPCR product. The PCR master mix was composed of 0.65µl 100pm of each primer (euro fins)(2.5 µl of 2.75Mm magnesium chloride, 2.5 µl of 10X PCR buffer, 1 µl of 20mM DNTP mixture and 0.2 µl 5U Tag DNA polymerase) (soils bio dye),16.5 µl sterilized double distilled water and 1 µl template DNA for 25 µl reaction Volume. PCR condition utilized to amplify the gene of interest was optimized as follows: initial denaturation at 94°C for 5minutes, followed by; 94°C as denaturation temperature for one minute, 65°C as annealing temperature for two minute and 72°C as an extension temperature one minute; for 35 cycles of amplification .The final extension temperature was 72 °C for 10°C minute. After PCR amplification the product was run in 1.5% of agrose gel and it was observed by gel documentation system.

4.7. Data Analysis

Statistical Analysis was performed with the SPSS (version, 20) package. Data like age, sex, residence, educational status, occupation were analyzed for possible associated risk factor. The risk of type I diabetics and *HLA-B 27* gene association was computed using chi- square test and odds ratio with confidence interval (CI 95%) which are interpreted as the relative risks of disease for T1DM. Allele frequencies were calculated by genotype counting, deviation from Hardy-Weinberg equilibrium was tested using a chi- square with one degree of freedom. Probability $(P) < 0.05$ was taken as the level of significance.

5. RESULTS

5.1. Demographics of study participant type one patients and control.

The demographic distribution of type one diabetes patients (n=100) and T1D sero-negative control participants (n=100) is present in Table one. Among the study patients the highest incidence of the disease was found among the age group < 40 years old which covers 48% of the study participants and the list affected age groups were greater than 75 years old. Among the100 type I diabetics patient 67 (67%) were male and the remaining were female. With respect to educational status and occupation the most affected patient groups were illiterate (43%) and unemployed (70 %), respectively. Around 75% of the type one diabetes patients came from rural keelson the other hand 56% of the control groups were in the age range of < 40 years old. With respect to sex 59% of the control groupware male, from educational points of view 39% of the controls were illiterate**,** when occupational status of the control group was tabulated 73% of were unemployed 27% were employed.

Table1: Demographic characters of study participants

Character	type one diabetes patients Case-groups (n=100)		type one diabetes sero- negative control groups(n=100)	
Risk factors	**Frequency**	**percent**	**Frequency**	**percent**
Age ≤ 40	**48**	**48**	**56**	**5**
40-75	**45**	**45**	**35**	**35**
≥ 75	**7**	**7**	**9**	**9**
Sex				
Male	**67**	**67**	**59**	**59**
Female	**33**	**33**	**41**	**41**
Residence				
Urban	**25**	**25**	**31**	**31**
Rural	**75**	**75**	**69**	**69**
Educati.st Illiterate	**43**	**43**	**39**	**39**
0-4	**18**	**18**	**21**	**21**
5-8	**34**	**34**	**30**	**30**
≥ 9	**5**	**5**	**10**	**10**
Occupation Employed.	**30**	**30**	**27**	**27**
un employed.	**70**	**70**	**73**	**73.**

5.2. The Association of demographic characters (risk factors) and type one diabetes disease.

Different risk factors (age, sex, residence, educational status, and occupation)were considered and analyzed for their risk factor for type one diabetes. Based on the result of the study age and residence has statistically significance risk associated with type one diabetic mellitus. As shown in Table 2 age group 40 years old (OR 1.370, P=0.0012) and 41-75 years old(OR=1.85, ,P=0.0031) were likely affected by type one diabetes in compassion with age group greater than 75 years old. The risks analysis also showed residence living in rural was at a higher risk for type one diabetes infection than rural.(OR=3.14.P=0.001).

Based on the result of this study sex (p=0.119) and educational states (0.0554) and occupation(p=0.0811).were not statistically associated to risk of type one diabetes militates disease in the study subjects (cases).

Table 2: Binary logistic regression analysis of risk factors for of T1D in the study participants

Risk Factors	β	P-value	O.R
0.000			
Age. ≤ 40	**.269**	**.41**	**1.370**
40-75	**.315**	**0.00**	**1.85**
Sex.			
Female	**369**	**.119**	**1.92**
Educati.st			
Illterate.	**874**	**.499**	**.190**
0-4	**.83.918**	**1.086**	
5-8	**.214**	**..691**	**1.23**
Residence			
Rurul.	**1.145**	**.001**	**3.14**
Occupation.			
Employed	**.854**	**.297**	**.426**

β=Beta value

5.3. The Association of HLA*B-27*.allele and demographic parameters

The association of *HLA-B27* allele and demographic parameters is given in Table 3 below. It was found that some of the demographic characteristics such as rural residence is found to have significance risk factor for the development of T1D patients the (p value 0.001). T1D patients that live in rural site were 68% more likely to development T1D as compared to urban residence (0R=3.15). Other factors such as sex, occupation and marital status did not show statistically significance association in developing T1D (p value 0 .369,0.297, 0.424), respectively.

The results of this study showed that, age and resedence have strongly assocation beteween *HLA B-27* geno trype and risks ofor type one diabetes disease development (p= 0.00.,..0.001),respectily. Howevet,sex, educational status ,and occupation did not show nosignificance associatin beteween*HLA B-27* geno trype and risks of for type one diabetes disease development (P=0.101,.357 and ,.811).

Table 3. Frequency of HLA B-27gene among study subjects (Note: P value; DF and x2 test were computedbySPSSversion8.(DF degree of freedom,*p<0.05)

Type one diabetes case		control groups				
Risk Factor	present	absent	present	absent	DF	X2 test p-value
	HLA B27%	HLA B27%	HLA B-27%	HLA B-27		
Age.						
≤ 40	37 (77.08%)	11 (22.91%)	15 (73.77%)	46(26.2%)	3	.000
40-75	26 (57.77%)	19(42.22%)	7 (62.5%)	22(37.5%)		
≥ 75	4(57.14%)	3(42%)	3(50%)	7(50%)		
Sex.						
Male.	41 (59.4%)	23 ((33.33%)	38(54.2%)	32(45.7)	1	.101
Female	.28 (77.77%)	8(25.80%)	12 (40%)	18(60%)		
Educational status						
Illiterate	31(72.02)	12(27.90%)	7(35%)	13 (65%)		
0-4	22 (64.7%)	12(35.29)	11(36 .6%)	19(63.33)3		.357
5-8	13 72,22%)	5(27.777%).	6(30%	14 (70%)		
≥ 9	3(60%)	2 (40%)	17(55,6)	13(54.4%)		
Residence						
Urban	11(37.93%)	18(62%)	17(37.7%)	28 (62.3 %)	1	.001
Rural	57 (80.5 %)	14(19.71%)	19(34.5%)	36 (63.5%)		
Occupants.						
Unempl.	51(72.8%)	19(27.14%)	23(45%)	27 (56%)		

Employed	**21(70%)**	**9(30%)**	**19(38%)**	**31 (62%)**	**1**	**.811**

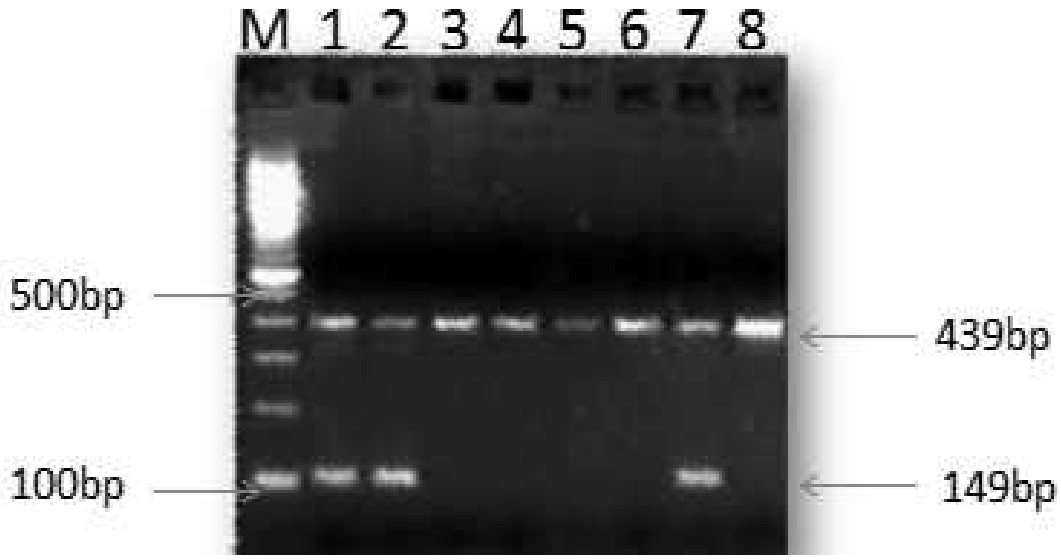

Figure 2. Representative agarose gel PCR amplification ofHLA-B27gene, Lane M=100bp DNA marker, Lanes 1,2,7 149bp. HLA B-27 mutant gene, lanes 3,4,5,6,8 are negative for HLA-B27

5.4. The frequency ofHLA*B-27*gene in Type one diabetes patients

After amplification a sharp band of 149 bps of amplified DNA was visualized under UV lights. Out of one hundred cases68 study subjects were positive and the remaining 32 cases were negative for HLA-B27 mutant allele. On the other hand, out of 100 control groups 75 individuals were negative and the remaining 25 individuals were positive for HLA-B27 mutant allele. There was statistically significance association between *HLA-B27* polymorphism ad risk of T1DM among cases (p=0.001, OR=3.14).

Table 4.The frequency of HLA B-27genotypes and allele in frequency in cases and controls of Type one diabetes patients

Study group	Genotype frequency (genotypes) Numbers of individuals	allele frequency	p-value
Cases	67%..67	.ooo	
Controls	25%	.25. . 001	

6. DISCUSSION

Several study with different population in the world described the presence of genetic alteration in the HLA gene. Indifferent population effects of these gene mutants were contradictory. Difference in susceptibility to type one diabetes due to population diversity based on SNPs (Single Nucleotide polymorphisms) in HLA gene may be result of divers evolutionary pressure between ethnicity as well as types of HLA and incidence of several forms of disease(Broning*et al.*,1996) and (Hakonarson*et al*.,2011). Type 1 diabetes is a polygenic disease which results from the interaction between environmental (viral, toxic, nutritional, socioeconomic (Dormant *et.al.*,1997) and genetic factors. It is the form of diabetes which occurs mainly in children and young adults (Bach,1989).Fortunately, molecular epidemiology offers the hope of the possibility of preventing the disease in the future, by evaluating the potential factors of risk of developing this pathology.

Besides, geographical location environmental condition and different socio demographic characteristics genetic behaviors of individuals have roles for diabetes mellitus disease and verity (Dfahlquistetall*et al*.,1998) .It is well established that associations between type 1 diabetes and certain HLA antigens largely facilitate the identification of the subjects having a potential risk to develop the disease.

According to the result of the present study all age groups and both sexes affected by type one diabetes mellitus disease. From the total type one diabetes mellitus patients 48 % were within the age range of $\leq$ 40 year old and 45%were from 41-75 years old. As compared to the females, males were highly affected by type one diabetes mellitus disease (67%) were females although there was no statistically significance difference between them($p = .119$). This result was consistent with health care in Ethiopia. which is carried out in area reputed the existence diabetes mellitus in all age group and both sex with high incidence in male 18.32 year old age group , similarly in a study that was carried out in the same place the prevalence of type one diabetes was higher in 30.4 year old .(Ayesha *et al.*, 2003).

Association between different demographic variation and type one diabetes disease were analyzing by binary logistic regression and the result is summarized in Table 3. The result of this study indicated that residence that is living in rural areas has shown relative increased risk for type one diabetes disease. Age < 40 years (OR = 3.14, p=0.001) was found to be a risk factor for diabetes mellitus disease as compared the age > 40 years old. This result is in consistent with another study doneSaudi which also have shown that age ,40 is a risk factor for diabetic militias one (Brown, 1998). The present study of *HLA –B27* polymorphism was determined through PCR-SSP techniques by using specific primer and control of human growth hormone. In this study among the 100 type one diabetes mellitus sero positive study subjects67 individual (67%) waspositive for *HLA-B27* mutant gene.

Therefore, there was statistically significance association between type one diabetes Miletus (P = 0.001) and *HLA-B 27* mutant gene. *HLA- B-27* is MHC class one molecule that is strongly associated with different type of diseases such as Ankylosing spondylitis and related spondyloarthritis. There is almost 90% association of *HLA-B27* with ankylosing spondylitis (Michael *et al*.2005).

Moreover, this gene polymorphism has been associated with risk of other diseases such as Psoriasis, Ankylosing spondylitis, Inflammatory bowel seas, and reactive arthritis(*Mitchell et al*., 2007).

The relationship between *HLA-B27* and many diseases has not yet been fully elucidated. Even if *HLA-B27* gene polymorphism has been associated in increasing many different type of disease, there is no any information in the literature that has been discussion the association of this gene with type one diabetics. As a result this makes difficult in discussing and comparing this finding with other similar studies.

7. CONCLUSION

In the present study, it is indicated that *HLA- B27* mutant allele has increased the risk of developing type one diabetic among study subjects. Moreover, there exist strong associated between the frequency of *HLA B-27*gene polymorphism age and inhabitancy towards increasing the risk of diabetic one among study subjects. However, it is early to call for the use of this allele as a marker for the risk of type one diabetic mellitus due to small sample size and inadequate data for comparison with other studies with similar and different genetic back grounds.

8. RECOMMENDATION

To have better treatment out comes molecular and genetic testing is vital and crucial. Therefore, engaging with similar type of study for diabetic and other metabolic disease that did not have great attention like communicable disease is an important instrument to reduce the number of working population that have acquired such metabolic diseases. To further use and recommend this gene polymorphism as a marker for early diagnosing of type one diabetic mellitus similar study with large sample size and different or similar study design is warranted. From the presentstudy it could be recommended that early diagnosis and intervention towards this disease, similar molecular studies on other metabolic genes is essential.

9. REFERANCE

Akerblom, HK.,Knip, M.,.andSimell. (1997). From pathomecanisms to prediction, prevention and improved care of insulin-dependent diabetes mellitus in children. Ann Med. **29:** 383-85

Ayesha, A., Motala, Mahomed, A., Omar, K., and Fraser, JP. (2003). Epidemiology of type 1 and type 2 diabetes in Africa. *J CardiovasRisk.*,**10**:77-83.

Bach, JF. (1989). L'origineimmunitairedu diabetes. La Recherché**214**:1206-1215

Bain, Prins, JB., and Hearne CM., *et al* .(2001). Insulin gene region-encoded susceptibility to type Diabetes is not restricted to HLA-DR4-positive individuals. Nat Genet: 2:212–215…

Bare, (1992).The desired outcomes of the medical management of diabetes include the.1022,1057

Bluestone, JA.,Herold .K , andEisenbarth, G., (2010).Genetics, pathogenesis and clinical interventions *in type l diabetes . Nature.**464** (7293): 1293–1300*

*Borch-Johnsen, K., Joner ,G., Mandrup-Poulsen, T., Christy, M., Zachau-Christiansen, B., Kastrup, K., Nerup, J., (November 1984). "Relation between breast-feeding and ncidence rates of insulin-dependent diabetes mellitus. A hypothesis". Lancet **2** (8411): 1083–6.*

*Brentjens, R.., andSaltz, L., (2001). "Islet cell tumors of the pancreas: the medical oncologist's perspective' SurgClin North Am (Review). **81** (3): 527–42. –*

BlajchmanMA., (1973).Histocompatibility (HLA) antigens, lymphocytotoxicymphocytotoxic antibodies and tissue antibodies in patients with diabetes mellitus. **22***: 429-432*

Brewerton, DA., Caffrerym.,Nicholsa , Walters D., and James, Dco (19730). Lancet **1**, 956-960. ,

Willett WC, Hu FB. Dietary fats and prevention of type2 diabetes". Progress in Lipid Research 48 (1): 44–51

Buehler, AM., Cavalcanti, AB.; Berwanger,O.;Figueiro M.; Laranjeira, LN.;and*et al*.(Jun 2013).Effect of tight blood glucose control versus conventional control in patients with type 2 diabetes mellitus: a systematic review with meta-analysis of randomized controlled trials. Cardiovascular **31** (3): *therapeutics*.147–60.

Brown, V., Alemu, S., Watkins, P., (1998). Diabetes in Ethiopia: overcomingthe problems of care delivery. *JDiabe .Nurs*.**2** (1):28-30.

Beck, S., Geraghty, D., Inoko H., Rowen, L.(1999). Complete sequenceand gene map of a human major Histocompatibilitycomplex.Nature .**401**: 921–23

Chang, Y.,Chuang, L. (2010). Review article the role of oxidative stress in the pathogenesis of type two diabetes: from molecular mechanism to clinicalimplication. *Am. J Transl.Res.,*2:316 331

Chiang, J., Kirkman M. S.; Laffel.L,. M., Peters A., L., (16 June 2014). Type 1 Diabetes Through *he Life Span: A Position Statement of the American Diabetes Association". Diabetes Care.**37** (7): 2034–2054.*

Daneman, D. (11 March 2006). "Type 1 diabetes".Lancet.**367** (9513): 847–58.

Devaraj, S, Glaser, N., Griffen, S., Wang-Polagruto, J., Miguelino, E., Jialal, I. (March 2006). "Increased Epidemiology of Insulin-dependent Diabetes Mellitus: WHO DiamonProject.GacMédMéx**l1:**151-. 154

Dorman, J., (1997).the WHO DiaMon Molecular Epidemiologie Sub-ProjectGroup: Molecular *activity and biomarkers of inflammation in patients with type 1 diabetes". Diabetes **55** (3): 774–779*

*Elliott,RB.,Pilcher, CC., Fergusson , DM., Stewart, AW. (1996). "A population based strategy to prevent insulin-dependent diabetes using nicotinamide". Journal of Pediatric Endocrinology and Metabolism.**9** (5): 501–9*

EspostiPujol-Borrell ,R., Vives-Pi, M., and, et al. (1996).Global gene expression changes in type 1 diabetes: insights into autoimmune response in the target organ and in the periphery. ImmunolLett 2010;133: 55–61

Fairweather, D., Rose, NR. (2002)."Type 1 diabetes: virus infection or autoimmune disease?".*Nat Immunol.**3** : 338–40.*

Feldtkeller, Ernst, Muhammad, Khan, van der Heijde, Desiree; van der Linden, Sjef, Braun, Jurgen (2003). *Age at disease onset and diagnosis delay in HLA-B27 negative vs. positive patients ithankylosing spondylitis . Rheumatology International **23:** (2): 61–66.*

Gill ,GV., Mbanya J., Ramaiya KL., Testate S., **(**2009). A Sub-Saharan African perspective of diabetes Diabetologia; **52**: 8–16)

*Galbraith W., Wagner MC., Chao J., Abaza M., Ernst LA, Nederlof MA., et al. (1991). "Imaging Cytometrybymultiparameter fluorescence". Cytometry**12** (7): 579–96..*

GonzelezGao, P., Seshasai,SR., and et al., *(2009).Daetes mellitus ,fasting blood glucose concenitration, and risk of vascular disease :A collaborative meta-analysis 102. prospective studiesSarwar ,**375** (9733): 2215–22.*

Helen ,Harkteader and Mary Ann Hogan**;** (2004). Fundamental of Nursing, Sander, 2nd ed, p (546-730).

*H u EA., Pan, A., Malik V,*an *Sun Q (2012) (Clinical research ed.)* ***344****: e1454*

Huxley, Rachel R; Peters, Sanne A E; Mishra, Gita D; Woodward, Mark (2015). "Risk of all-cause mortality and vascular events in women versus men with type 1 diabetes: a systematic review and meta-analysis". The Lancet Diabetes and Endocrinology **3**: 198–206.

Isomaa , Albert , E., Conrad, M., Keller, E., Hummel, M., Ferber , K., et al (1999.) IDDM2 /insulin VNTR modifies risk conferred by IDDM1/HLA for development of Type 1 diabetes and associatedautoimmunity. Diabetologia;**46**:712–20

Kitabchi, AE.,Umpierrez, GE.; Miles, JM.; Fisher, JN (Jul 2009). "Hyperglycemic crises in adult patients with diabetes.". Diabetes Care ***32*** *(7): 1335–43.*

*Knip, M., Veijola, R., Virtanen, SM., Hyöty, H., Vaarala, O., Akerblom, HK. (2005). "Environmental Triggers and Determinants of Type 1 Diabetes".Diabetes.****54****: S125–S136.*

Kida, K., Kaino, Y., Ito, T., Hirai, H., Nakamura, K.(1999).Immuno genetics of insulin-dependent diabetesmellitus. ActaPædiatr, Supp**l427:**3-7

Khan, M. (2010)."HLA and spondyloarthropathies".In Narinder K. Mehra. The HLA omplex *in Biology and Medicine. New Delhi, India: Jayppee Brothers Medical Publishers. pp. 259–275*

Lee, IM. , Shiroma, EJ.,Lobelo, F., Puska, P., Blair, SN., Katzmarzyk, PT. (2012). Lancet Physical ActivitySeries Working ***380****:219-229.*

Lewis, R.W.(1996).Epidemiology of erectile dysfunction. Urologic Clinics of Type one diabetes mellitus in childhood: a matched case control study in Lancashire The Lancet Diabetes and Endocrinology **3**: 298–306.

McCance, DR., Hanson, RL.,Pettitt, DJ., Bennett, PH., Hadden, DR., Knowler WC.(1997) Diagnosing diabetes mellitus – do we need new criteria? *Diabetologia*; **40:** 247–55.

Michael, T., Seipp, (2005).Maria PCR Melting Assay and Two Flow Cytometric Antigen Assays, Cytometry, Part B (Clinical Cytometry), 63B, 10–15.

Malik, VS., Popkin, BM., Bray, GA., Després, JP., Hu, FB. (2010-03-23). Circulation ***121*** *(11):1356–64.*33

Motala, A., and Ramaiya, K., (2010.) Diabetes: the hidden pandemic and its impact on sub-Saharan Africa. Diabetes Leadership Forum. Available**24** 369-375.

Njolstad,PR., Sagen ,JV., Bjorkhaug ,L., Odili, S., and ShehadehN. (2003). Permanent Neonatal diabêtes caused byglucokinase deficiency: inbor error of the glucose-insulin signaling pathway. Availab NIDDK, May : (2015).Retrieved 31 July 2016.

NIDDK.July:(2016) Retrieved 31 July 2016.
Peter, H. (2007) Understanding diabetes.1th*Edition.MD*; 978- 987
Petzold, A.; Solimena, M.; Knoch ,KP., (2015)."Mechanisms of Beta Cell Dysfunction Associated *With Viral Infection.".CurrDiab Rep (Review).**15**: 73*
phipps, Payne, F., Lowe CE, Hermann R, Healy BC, Harold D, et al.(1987) Remapping the insulin gene/IDDM2 locus in type 1 diabetes. Diabetes;53:1884–9
Rachel ,R.; Peters, Sanne, A E.; Mishra, Gita D.; Woodward, Mark (2015). "Risk of all-cause Mortality and vascular events in women versus men with type 1 diabetes: a systematic review and meta ,analysis".The Lancet Diabetes and Endocrinology **3**: 198–206.
*Risérus U., Willett , WC., Hu FB.,(2009). "Dietary fats and prevention of type 2 diabetes".*Xenical,,(1995). New Hope for the Treatment of Obesity **7**:1
Roe, B., Crabtree, J., .and khan, A., Protocols for recombinant DNA Isolation ,cloning and sequencing (Internet edition) .university of oklaome, Norman. Available at http://www.genome. Ou.edu/protocol book protocol index.html.(accessed on 27/11/2014).
Rother, KI., (April 2007). "Diabetes treatment bridging the divide".*The New England Journal of Medicine **356** (15): 1499–501.*
Royle, J., and Walsh M., (1992): Watson's Medical-. Surgical Nursing and Related Physiology,1992.4th.ed,. 0-7020-1515-1516.
Schlosstein ,P., Terasaki ,R., Bluestone C., and Pearson: (1973) .High association of an HLA a*ntigen C* W2 with ankylosingspondylitis, *The New England Journal of Medicine*, **288** (14):704-7046.
Shai I., Jiang R, Manson JE, Stampfer, MJ., Willett, WC, ColditzGA, Hu FB. Ethnicity, Obesity, and Risk of type 2 Diabetes in Women Diabetes Care, 2006, 29:1585–1590.
*Shehadeh N, Shamir R, Berant M, Etzioni A (2001). "Insulin in human milk and the prevention of type 1 diabetes". Pediatric Diabetes **2** (4): 175–7.*
Shoback, edited by David G. Gardner, Dolores (2011)."Chapter 17". Greenspan's basic and clinical endocrinology (9th ed.). New York: McGraw-Hill Medical. ISBN 0-07-162243- 8.
Smeltzer,and Bare, (1992).The desired outcomes of the medical management of diabetes include the.1022,1057
Smeltze rand Bare, (1992).The desired outcomes of the medical management of diabetes include the. 1022,1057.
Singal, DP, Blajchman MA.(1973)Histocompatibility (HL-A) antigens, lymph cytotoxic

antibodies and tissue antibodies in patients with diabetes mellitus. Diabetes. Jun;22(6):429–432

Sylvain K., Aurelie H., Marc M, Christophe R , (2004).Rapid screening for HLA-B27 by a Taqman-PCR assay using sequence specific primers and a minor grove binding probe, a novel type of Taqmaprobe.*JImmunol Methods*, **287**, 179-186...

Thayer KA., HeindelJJ., Bucher JR., Gallo MA., : (Jun 2012). "Role of environmental chemicals in diabetes and obesity: a National Toxicology Program workshop review". Environ Health Prospect (Review).**120** (6): 779–89

Toljamo ,Todd JA, Walker NM, Cooper JD, Smyth DJ, Downes, K., Plagnol, V, et al.(2001). Robust associations of four new chromosome regions from genomewideanalyses of type 1 diabetes. Nat Genet;39:857–64.Brewerton

Veijola R., Virtanen SM., Hyöty H., Variable O., and Akerblom,HK., (2005). "Environmental *Triggers and Determinants of Type 1 Diabetes".Diabetes.**54**: S125–S136.*

Whitehorse, Todd, JA, Nadeau, J., Grabs, R., Goodyer, CG.,*et al*. (2002). Insulin expression in human thymuses modulated by INS VNTR alleles at the IDDM2 locus. Nat Genet 1997;15:289–92.

WHO. October (:2013). Retrieved 25 March 2014.

WHO. June (2016).Archived from the original on 26 August 2013.*Retrieved* 31 July 2016.

Virtanen, SM., Knip, M., (December 2003)

WHO. October 2013. Archived from the original on 26 Aug 2013.Retrieved 25 March 2014.

Wu, J., Yan , LJ,. (2015). Streptozotocin-induced type 1 diabetes in rodents as a model for study**:**181 188

Yajnik, CS., Shelgikar, KM., Naik SS. Kanitkar ,SV.,andOrskovH.(1992) The KetoacidosisresistanceInfib–calculous–pancreatic–diabetes.DiabetesRes ClinPract**15** 149–56.

Appendix

Annex 1

University of Gondar

College of Natural and Computational Sciences

Department of Biotechnology

The English version of the Consent Form

Title of The research Association of Human leukocyte antigen (HLA*) B-27* polymorphism and risk of diabetic type 1 among patients visiting Gondar University teaching hospital

1. The undersigned----------Who is living at ----------zone--------wereda--------kebele and-------Town Willingly, agree to provide 3 ml of my blood sample for the principal investigator of this research .the investigators should ethically bind and use this blood sample only for the said research and will completely be responsible if you don't keep the result and states of the sample confidential.

Name and signature of the participant

Date.

---------- ----------.

Annex 2 *The Amharic version of the Consent Form)*

ጎንደርዩኒቨርስቲ

የተፈጥሮናቀመርሳይንስኮሊጅ

የባዮተክኖሎጅት/ክፍል

የምርምሩርዕስ፡**The research Association of Human leukocyte antigen (HLA*) B-27* polymorphism and risk of diabetic type one among patients visiting Gondar University teaching hospital**

እኔ_____የተባልኩትግለስብ በ----------ዞንበ---------- ወረዳበ--------ቀበሌእናበ ----------ከተማየምኖር

መስሆን፤3

ሚሊየሚሆንደማንለናመናለመስጠትስስማማ፤የሰጠሁትደምከተባለዉአገልግሎትዉጭእንደማይዉልናከተባለዉዉጭ

ለሚሰሩስራዎችምርምሩንየሚያካፈሉሰወችሀላፊነትእነደሚወስዱበመተማመንነዉ።።

የተሳታፊዉስምናፊርማቀን

Annex 3

University of Gondar

College of Natural and Computational Sciences

Department of Biotechnology

Title ofThe research Association of Human leukocyte antigen (HLA*) B-27* polymorphism and risk of diabetic type 1 among patients visiting Gondar University teaching hospital

Questionnaire to be filled by physician/nurse/laboratory technician by interviewing the patien

*Names of hospital health center*_____*sample no.* _____

*Age*_____ *sex*_____

Locality_____

Educationnal Status. _____

Marital status,_____

Occupation._____

Name and signature of the *physician/nurse/laboratory technicion*_____.

_____